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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Michael Giesing et al.
Application No.	:	09/485,879
Filed	:	June 22, 2000
For	:	METHOD FOR THE CHARACTERIZATION OF DISSEMINATED AND MICROMETASTASIZED CANCER CELLS

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Examiner	:	Jeanine Enewold Goldberg
Art Unit	:	1655
Docket No.	:	790076.401
Date	:	March 13, 2001

Commissioner for Patents
Washington, DC 20231

AMENDMENT

Commissioner for Patents:

In response to the Office Action dated September 13, 2000, please extend the period of time for response three months, to expire on March 13, 2001. Enclosed are a Petition for an Extension of Time and the requisite fee. Please amend the application as follows:

In the Abstract:

Please delete the abstract of the disclosure and replace it with the following revised abstract, which does not contain more than 250 words:

--The invention relates to a diagnostic method and related compositions for characterizing disseminated and micrometastasized cancer cells in body fluids using DNA, mRNA

and/or protein. According to the method, DNAs, mRNAs and/or proteins of cancerous and non-cancerous cells obtained from a body fluid (*i.e.,* cell preparations that are not enriched for cancer cells) and DNAs, mRNAs and/or proteins of cancerous cells that are removed from the body fluid (*i.e.,* cell preparations that are enriched for cancer cells) are analyzed, to determine the presence or expression in body fluid cells of at least one cancer-specific gene that is not expressed in non-cancer cells or of at least one cancer-associated gene, and of at least one cancer-specific or cancer-associated gene in cancer cells removed from the body fluid cells (*i.e.,* in isolated cancer cells). Also provided are compositions and methods for testing antineoplastic substances and anticancer therapies by characterizing a cancer-specific or a cancer-associated gene, and examples of cancer-specific and cancer-associated genes, including tissue-specific genes, tumor suppressor genes, oncogenes and others.--

In the Claims:

Please cancel claims 1-17.

Please add the following new claims.

--18. (new) A method for determining a risk for or presence of a disseminated cancer cell or a micrometastasizing cancer cell in a body fluid from a subject known to have or suspected of being at risk for having a disseminated cancer cell or a micrometastasized cancer cell, comprising:

detecting, in a plurality of cells obtained from a body fluid of a subject known to have or suspected of being at risk for having a disseminated cancer cell or a micrometastasized cancer cell, an absence or presence of at least one nucleic acid selected from the group consisting of a first cancer-specific nucleic acid and a first cancer-associated nucleic acid, wherein said plurality of cells comprises at least one cancer cell and at least one non-cancer cell; and

detecting, in at least one cancer cell removed from said plurality of cells, the absence or presence of at least one nucleic acid selected from the group consisting of a second cancer-specific nucleic acid and a second cancer-associated nucleic acid, wherein said first and second cancer-specific nucleic acids are different, wherein said first and second cancer-associated nucleic acids are different and wherein an increased presence of said nucleic acids in said cancer cell relative

to the presence or absence of said nucleic acids in said non-cancer cell indicates an increased risk for having a disseminated cancer cell or a micrometastasized cancer cell.

19. (new) A method for determining a risk for or presence of a disseminated cancer cell or a micrometastasizing cancer cell in a body fluid from a subject known to have or suspected of being at risk for having a disseminated cancer cell or a micrometastasized cancer cell, comprising:

detecting an absence or presence of at least one first cancer-specific nucleic acid in a plurality of cells obtained from a body fluid of a subject known to have or suspected of being at risk for having a disseminated cancer cell or a micrometastasized cancer cell, said plurality of cells comprising at least one cancer cell and at least one non-cancer cell; and

detecting, in at least one cancer cell removed from said plurality of cells, the absence or presence of at least one second cancer-specific nucleic acid, wherein said first and second cancer-specific nucleic acids are different and wherein an increased presence of said nucleic acids in said cancer cell relative to the presence or absence of said nucleic acids in said non-cancer cell indicates an increased risk for having a disseminated cancer cell or a micrometastasized cancer cell.

20. (new) A method for determining a risk for or presence of a disseminated cancer cell or a micrometastasizing cancer cell in a body fluid from a subject known to have or suspected of being at risk for having a disseminated cancer cell or a micrometastasized cancer cell, comprising:

detecting an absence or presence of at least one first cancer-specific nucleic acid in a plurality of cells obtained from a body fluid of a subject known to have or suspected of being at risk for having a disseminated cancer cell or a micrometastasized cancer cell, said plurality of cells comprising at least one cancer cell and at least one non-cancer cell;

detecting, in at least one cancer cell removed from said plurality of cells, the absence or presence of at least one second cancer-specific nucleic acid, wherein an increased presence of said cancer-specific nucleic acids in said cancer cell relative to the presence or absence of said cancer-specific nucleic acids in said non-cancer cell indicates an increased risk for having a disseminated cancer cell or a micrometastasized cancer cell; and

detecting an absence or presence of at least one cancer-associated nucleic acid in at least one cell in one or more samples selected from the group consisting of (i) a plurality of cells obtained from a body fluid of a subject suspected of being at risk for having a disseminated cancer cell or a micrometastasizing cancer cell, said plurality of cells comprising at least one cancer cell and at least one non-cancer cell, and (ii) at least one cancer cell removed from said plurality of cells obtained from a body fluid of a subject suspected of being at risk for having a disseminated cancer cell or a micrometastasizing cancer cell, said plurality of cells comprising at least one cancer cell and at least one non-cancer cell,

wherein an increased presence of said cancer-associated nucleic acid in said cancer cell relative to the presence or absence of said nucleic acid in said non-cancer cell indicates an increased risk for having a disseminated cancer cell or a micrometastasized cancer cell.

Sub C1 21. (new) The method of claim 20 wherein the first and second cancer-specific nucleic acids are the same.

G2

22. (new) The method of claim 20 wherein the first and second cancer-specific nucleic acids are different.

23. (new) The method of any one of claims 18-20 wherein the nucleic acid is selected from the group consisting of DNA and RNA.

Sub C2 24. (new) The method of claim 23 wherein the RNA comprises mRNA.

25. (new) The method of claim 24 wherein the mRNA is not expressed in the non-cancer cell.

26. (new) The method of claim 25 wherein the mRNA comprises all or a portion of a transcript of a gene selected from the group consisting of a CEA gene, a CK20 gene, a MUC1 gene, a tyrosinase gene and a MAGE3 gene.

Sub C3>

27. (new) The method of claim 23 wherein the DNA that is detected comprises genomic DNA selected from the group consisting of genomic DNA comprising a genomic mutation, genomic DNA comprising a gene that has undergone amplification, genomic DNA comprising a gene that has undergone loss of heterozygosity, genomic DNA comprising a translocated gene and genomic DNA comprising a gene polymorphism.

28. (new) The method of claim 23 wherein at least one nucleic acid that is detected comprises DNA, said DNA comprising genomic DNA selected from the group consisting of (i) the second cancer-specific nucleic acid and (ii) a cancer-associated nucleic acid that is present in at least one cancer cell removed from the plurality of cells and that is absent from any non-cancer cells of the plurality of cells.

C2

29. (new) The method of claim 23 wherein the DNA is genomic DNA that comprises all or a portion of an oncogene.

30. (new) The method of claim 23 wherein the DNA is genomic DNA that comprises all or a portion of a tumor suppressor gene.

31. (new) The method of claim 27 wherein the genomic DNA comprises all or a portion of a gene selected from the group consisting of a p53 gene, an erb-B2 gene, a c-myc gene, a K-ras gene, an RB gene, an APC gene and a DCC gene.

Sub C4>

32. (new) The method of any one of claims 18-20 wherein at least one nucleic acid selected from the group consisting of a cancer-specific nucleic acid and a cancer-associated nucleic acid comprises a coding portion of a gene selected from the group consisting of a tissue-specific gene, a metastasis-associated gene, a steroid hormone receptor gene, a drug resistance gene, an immunomodulation gene, a cell proliferation gene and an apoptosis gene, or a complementary nucleic acid thereto.

33. (new) The method of claim 32 wherein the metastasis-associated gene encodes a gene product selected from the group consisting of an angiogenesis factor, a motility factor, a growth factor, a matrix degradation factor and an adhesion factor.

34. (new) The method of claim 33 wherein the matrix degradation factor is selected from the group consisting of a proteinase and a proteinase inhibitor.

35. (new) The method of claim 33 wherein the adhesion factor is an adherin.

36. (new) The method of claim 24 wherein the mRNA encodes a gene product selected from the group consisting of bFGF, bFGF-R, VEGF, VEGF-R1, VEGF-R2, MMP2 and TIMP3.

Cl 2 sub C5>

37. (new) The method of any one of claims 18-20 wherein the cancer cell is removed from the plurality of cells by a method selected from the group consisting of microfiltration, density gradient centrifugation and antigen-specific immunoadsorption.

38. (new) A method for identifying an anticancer therapy, comprising:

(a) detecting, before and after administering a candidate anticancer therapy to a subject known to have or suspected of being at risk for having a disseminated cancer cell or a micrometastasized cancer cell,

(i) in a plurality of cells obtained from a body fluid of the subject, an absence or presence of at least one nucleic acid selected from the group consisting of a first cancer-specific nucleic acid and a first cancer-associated nucleic acid, said plurality of cells comprising at least one cancer cell and at least one non-cancer cell; and

(ii) in at least one cancer cell removed from said plurality of cells, the absence or presence of at least one nucleic acid selected from the group consisting of a second cancer-specific nucleic acid and a second cancer-associated nucleic acid, wherein said first and second cancer-specific nucleic acids are different, and wherein said first and second cancer-associated nucleic acids are different; and

(b) determining, after administering the candidate anticancer therapy, a decreased presence of said nucleic acids in said cancer cell relative to the presence or absence of said nucleic acids in said non-cancer cell, and therefrom identifying an anticancer therapy.

39. (new) A method for identifying an anticancer agent, comprising:

(a) detecting in at least one cell, before and after contacting a candidate anticancer agent with a plurality of cells known to include or suspected of including a disseminated cancer cell or a micrometastasized cancer cell,

(i) an absence or presence of at least one nucleic acid selected from the group consisting of a first cancer-specific nucleic acid and a first cancer-associated nucleic acid, and

(ii) in at least one cancer cell removed from said plurality of cells, the absence or presence of at least one nucleic acid selected from the group consisting of a second cancer-specific nucleic acid and a second cancer-associated nucleic acid, wherein said first and second cancer-specific nucleic acids are different, and wherein said first and second cancer-associated nucleic acids are different; and

(b) determining, after contacting the candidate anticancer therapy with the cells, a decreased presence of any one or more of said nucleic acids in said cancer cell relative to the presence or absence of said nucleic acids in a non-cancer cell, and therefrom identifying an anticancer agent.

40. (new) A method of typing a malignant disease in a subject known to have, or suspected of being at risk for having, a malignant disease, comprising:

detecting an absence or presence of at least one first cancer-specific nucleic acid in a plurality of cells obtained from a body fluid of a subject known to have or suspected of being at risk for having a disseminated cancer cell or a micrometastasized cancer cell, said plurality of cells comprising at least one cancer cell and at least one non-cancer cell; and

detecting, in at least one cancer cell removed from said plurality of cells, the absence or presence of at least one second cancer-specific nucleic acid, wherein said first and second cancer-specific nucleic acids are different and wherein an increased presence of said nucleic acids in said cancer cell relative to the presence or absence of said nucleic acids in said non-cancer cell indicates a type of malignant disease from which the cancer cell is derived.--

REMARKS

Reconsideration of the present application in view of the present amendment and the following remarks is respectfully requested. The application is amended to include a new abstract of not more than 250 words, replacing the abstract of the original application. New claims 18-40 submitted herewith by amendment are pending. Applicants hereby cancel claims 1-17 without prejudice to the filing of any divisional, continuation, or continuation-in-part application. Support for claims 18-40 may be found in the specification, by way of non-limiting example, at page 4, lines 24-34; page 5, line 15 through page 6, line 6; page 6, line 25 through page 7, line 17; page 9, line 31 through page 10, line 25; page 15, line 11 through page 16, line 30; page 17, line 18 through page 18, line 22 (*e.g.,* for a description of "cells obtained from a body fluid" as contrasted with a "cancer cell removed from" the body fluid); page 21, lines 9-19; page 26, line 1 through page 27, line 8; in the Examples (pages 31-65) and elsewhere. No new subject matter has been added.

The present application is directed in part to a method for determining a risk for or presence of a disseminated cancer cell or a micrometastasizing cancer cell in a body fluid from a subject known to have or suspected of being at risk for having a disseminated cancer cell or a micrometastasized cancer cell, comprising detecting, in a plurality of cells obtained from a body fluid of a subject known to have or suspected of being at risk for having a disseminated cancer cell or a micrometastasized cancer cell, an absence or presence of at least one nucleic acid selected from a first cancer-specific nucleic acid and a first cancer-associated nucleic acid, wherein the plurality of cells comprises at least one cancer cell and at least one non-cancer cell; and detecting, in at least one cancer cell removed from the plurality of cells, the absence or presence of at least one nucleic acid selected from a second cancer-specific nucleic acid and a second cancer-associated nucleic acid,

wherein the first and second cancer-specific nucleic acids are different, wherein the first and second cancer-associated nucleic acids are different and wherein an increased presence of the nucleic acids in the cancer cell relative to the presence or absence of the nucleic acids in the non-cancer cell indicates an increased risk for having a disseminated cancer cell or a micrometastasized cancer cell.

Applicants respectfully submit that the present invention therefore provides the surprising advantages conferred by determining a risk for or presence of a disseminated cancer cell or a micrometastasizing cancer cell according to the *two* steps of (i) detecting at least one cancer-specific or cancer-associated nucleic acid or gene (*e.g.,* genomic DNA) or cancer-specific or cancer-associated nucleic acid or gene product (*e.g.,* mRNA) in a first sample comprising a plurality of "cells obtained from a body fluid" of a subject (as described in the specification, for example, at page 17, lines 18-32), which cells include normal and cancerous cells, *and* (ii) further detecting at least one additional cancer-specific or cancer-associated nucleic acid or gene or gene product in a second sample derived from the first sample and comprising a cancer cell "removed" from the body fluid (as described in the specification, for example, at page 17, line 34 through page 18, line 22). The invention is thus directed in pertinent part to the unexpected advantages which result from determining at least two distinct nucleic acids or genes, including the use of a greater number of markers as described in the Examples. Additionally, as disclosed in the specification and recited in the claims, removal of the cancer cell from the body fluid preparation may be achieved by a variety of methods known to the art, including, for instance, microfiltration, density gradient centrifugation or antigen-specific immunoadsorption. As also described in the specification, such removal provides a fraction of the cells that are initially present in the body fluid preparation, which fraction comprises isolated and/or enriched cancer cells.

The relative presence or absence of the cancer-specific or cancer-associated nucleic acid, gene or gene product in *positively selected* cancer cells is thus determined according to step (ii) described above and, if increased when compared to the level of the cancer-specific nucleic acid, gene or gene product in the unfractionated body fluid cell preparation, indicates an increased risk for the presence of a disseminated cancer cell or a micrometastasized cancer cell, such as an increased metastatic risk (see, *e.g.,* specification Examples at pages 51-65). Based on the instant disclosure, a person having ordinary skill in the art will readily appreciate what is a "cancer-specific" gene (*e.g.,* specification at page 5, line 30 through page 6, line 23). As noted above, the invention method also

comprises detecting and comparing levels of a "cancer-associated" gene (i) in unfractionated body fluid cells and (ii) in *positively selected* cancer cells, *i.e.,* cancer cells that have been "removed" from the body fluid as discussed above. "Cancer-associated" genes are described in the specification, for example, at page 5, line 30 through page 6, line 23. As described in the specification (*e.g.,* at page 6, line 25 through page 7, line 10; *see also* source references for the various recited genes, which are provided at pages 65-92) and recited in the claims, cancer-specific and cancer-associated genes or gene products are preferably detected as nucleic acids, for instance, as DNA (*e.g.,* genomic DNA) or as RNA (*e.g.*, mRNA).

Turning now to the Office Action, the Examiner correctly states that the present application is a national stage application under 35 U.S.C. §371 that has been converted from PCT application number PCT/EP98/05360, which has an international filing date of August 24, 1998. Applicants respectfully note that the Action misstates the priority claim of PCT/EP98/05360; that PCT application claims priority to DE 197 36 691.0, which was filed on August 22, 1997. The first paragraph of the Action states that a translation of this priority document has not been provided. It is applicants' understanding pursuant to 37 C.F.R. §1.495(f) that translation of this priority document may be required where such translation is considered necessary. However, where the art cited in the Action predates the claimed priority filing date of DE 197 36 691.0, it is not clear to applicants that a translation is necessary at this time. Accordingly, applicants respectfully request clarification from the Examiner regarding the reference to the priority document in the first paragraph of the Action, and submit that a translation can be provided in the future should a requirement for same be identified at a later date.

The Examiner objected to the abstract of the disclosure, asserting that it contains more than 250 words in contravention of MPEP §608.01(b). The application has been amended to include a revised abstract of not more than 250 words as a replacement for the original abstract, and now complies with the cited requirement.

The Examiner objected to claims 6-13 under 37 C.F.R. §1.75(c) as being in improper form because a multiple dependent claim (claim 6) depends upon another multiple dependent claim (claim 5). Applicants respectfully submit that the basis for this objection has been rendered moot in view of the present amendment.

REJECTIONS UNDER 35 U.S.C. §101

Claims 14 and 15 are rejected under 35 U.S.C. §101 as allegedly being directed to non-statutory subject matter. In particular, the Action asserts that these claims improperly define a process where the recitation "use" is not accompanied by any setting forth of steps involved in the process. Applicants respectfully submit that these grounds for rejection have been obviated by the present amendment, and therefore request that the rejection be withdrawn.

REJECTIONS UNDER 35 U.S.C. §112, SECOND PARAGRAPH

Claims 1-17 are rejected under 35 U.S.C. §112, second paragraph, for indefiniteness. More specifically, the Action asserts that claims 1-17 do not recite a positive process that clearly relates back to the preamble, and that the metes and bounds of the invention are unclear where the relationships are ambiguous between two recited method steps, and between "cells obtained from body fluid" and "cancer cells removed from body fluid". The Examiner is also unclear with regard to the meanings of the recitations "multiple characterization of disseminated and micrometastasized cancer cells", "cancer-specific gene which is essentially not expressed in non-cancer cells", "the genomic DNA", "investigate", "such as", "are also investigated singly" and "means for carrying out the method".

Applicants respectfully traverse these grounds for rejection, and submit that the present application as amended satisfies all requirements of 35 U.S.C. §112, second paragraph. The meanings of "cells obtained from body fluid" and "cancer cells removed from body fluid" are abundantly clear, as described in the specification, for example, at page 17, line 18 through page 18, line 22, and as discussed above. Accordingly, and as also discussed above, the application is directed in pertinent part to a method for determining a risk for or presence of a disseminated cancer cell or a micrometastasizing cancer cell in a body fluid from a subject known to have or suspected of being at risk for having a disseminated cancer cell or a micrometastasized cancer cell, according to which method a cancer-specific nucleic acid and/or a cancer-associated nucleic acid is detected (i) in unfractionated cells obtained from a body fluid *and* (ii) in positively selected cancer cells that have been physically removed (*e.g.*., isolated cancer cells) from the body fluid cells. Applicants therefore respectfully submit that the rejections under 35 U.S.C. §112(2) directed to "cells obtained from a

body fluid" and to a "cancer cell removed from" the body fluid have been overcome, and that any remaining grounds for rejection under 35 U.S.C. §112(2) have been rendered moot by the present amendment. Accordingly, applicants therefore respectfully request that these rejections be withdrawn.

REJECTIONS UNDER 35 U.S.C. §102(B)

Claims 1-17 are rejected under 35 U.S.C. §102(b) as each being anticipated by at least one of Selby et al. (WO 96/17080), Pantel et al. (1995 *Onkologie* 18:394-401), Jung et al. (1997 *Eur. J. Clin. Chem. Clin. Biochem.* 35:3-10), Schmitz-Drager et al. (1996 *World J. Urol.* 14:190-196), Noguchi et al. (1994 *Cancer* 74:1595-1600) and Pelkey et al. (1996 *Clin. Chem.* 42:1369-1381). More specifically, the Examiner alleges that Selby et al. teach detection of CK20 mRNA in several carcinomas of epithelial origin but not in normal blood, that Pantel et al. teach cancer detection using DNA and RNA from several tumor-associated or tissue-specific genes, that Jung et al. teach RT-PCR detection of several cancer-specific genes in samples from human solid tumors, that Schmitz-Drager et al. teach oncogene and tumor suppressor gene detection in tumors having high metastatic potential, that Noguchi et al. teach MUC1 mRNA detection by RT-PCR in breast carcinoma micrometastases, and that Pelkey et al. teach RT-PCR detection of several cancer-associated genes which correlate with micrometastases in cells obtained from body fluid.

Applicants respectfully traverse these grounds for rejection, and submit that none of the cited references teaches or suggests the presently claimed invention. As noted above, the present invention is directed in pertinent part to a method for determining a risk for or presence of a disseminated cancer cell or a micrometastasizing cancer cell in a body fluid from a subject known to have or suspected of being at risk for having a disseminated cancer cell or a micrometastasized cancer cell, comprising detecting an absence or presence of at least one first nucleic acid which is a cancer-specific and/or cancer-associated nucleic acid in a plurality of cells obtained from a body fluid of a subject known to have or suspected of being at risk for having a disseminated cancer cell or a micrometastasized cancer cell, the plurality of cells comprising at least one cancer cell and at least one non-cancer cell; *and* detecting, in at least one cancer cell *removed* from the plurality of cells, the absence or presence of at least one second, distinct nucleic acid which is a cancer-specific and/or a cancer-associated nucleic acid, wherein an increased presence of the detected nucleic acids

in the cancer cell relative to the presence or absence of the nucleic acids in the non-cancer cell indicates an increased risk for having a disseminated cancer cell or a micrometastasized cancer cell.

Applicants submit that none of the cited references discloses the present invention, where it is well settled that in order for a reference to anticipate a claimed invention, each and every element recited in the claim must be present in a single reference. The prior art fails to provide such a reference, and the Action fails to point distinctly to every claim limitation within any one of the cited publications. In particular, and as discussed above, none of the references cited in the Action teaches a method for determining risk for having a disseminated cancer cell or a micrometastasized cancer cell that comprises detection of at least one first cancer-specific and/or cancer-associated nucleic acid in an unfractionated (*i.e.,* heterogeneous with respect to cancer and non-cancer cells) population of cells obtained from a body fluid, *and* that further provides the step of detecting at least one second, distinct cancer-specific and/or cancer-associated nucleic acid in a cancer cell subpopulation that has been selectively enriched or isolated (*e.g.,* "removed") from such a starting cell population. In this regard, Selby et al. merely disclose detection of CK20 in a sample believed not to normally express this marker, but Selby et al. fail to teach detecting CK20 or any other cancer-specific or cancer-associated marker in a cancer cell that has been removed from the sample. Pantel et al. merely provide a review of immunocytochemical, enzymatic and nucleic acids-based detection of tumor-associated molecular markers, but these authors fail to disclose a method for determining a risk for having a disseminated cancer cell or a micrometastasized cancer cell by detecting a first cancer-specific and/or cancer-associated nucleic acid in cells obtained from body fluid *combined with* detecting a second, distinct cancer-specific and/or cancer-associated nucleic acid in a cancer cell removed from such cells. In like manner, Jung et al., Schmitz-Drager et al., Noguchi et al. and Pelkey et al. all also fail to teach or suggest the subject invention method, where these references fail to disclose detection of at least one first cancer-specific or cancer-associated nucleic acid in an unfractionated cell preparation obtained from a body fluid *combined with* detection of a second, distinct cancer-specific or cancer-associated nucleic acid in a cancer cell that has been removed from such cells. Accordingly, applicants respectfully submit that in view of the present amendment, the Action has failed to establish anticipation by the prior art of any currently pending claim. Applicants therefore believe that the present application satisfies the requirements of 35 U.S.C. §102(b).

All of the claims remaining in the application are now clearly allowable. Favorable consideration and a Notice of Allowance are earnestly solicited.

Respectfully submitted,

Michael Giesing et al.

SEED Intellectual Property Law Group PLLC

Stephen J. Rosenman, Ph.D.
Registration No. 43,058

Enclosures:

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701 Fifth Avenue, Suite 6300
Seattle, Washington 98104-7092
Phone: (206) 622-4900/ Fax: (206) 682-6031
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